Male genitalia's evolutionary rate is higher than those of body traits: the case of two *Liolaemus* lizards' group

A. M. Quipildor[1] (iD), M. R. Ruiz-Monachesi[1] (iD), S. Ruiz[1], T. N. Hibbard[1], S. Valdecantos[1,2] & F. Lobo[1,2]

[1]CONICET- Instituto de Bio y Geo Ciencias del NOA (IBIGEO), Salta, Argentina
[2]Anatomía comparada, Facultad de Ciencias Naturales, Universidad Nacional de Salta (UNSa), Salta, Argentina

Abstract

Different studies suggest that reproductive characters evolve faster than non-reproductive characters. Males in the order Squamata have paired copulatory organs called hemipenes, with high morphological diversity, including differences in size, shape, and ornamentation. Some studies in the species-rich lizard genus, *Anolis* suggest that genital traits evolve faster than the rest of the body. However, these studies were made considering only a few traits, across a wide phylogeny, without considering species relatedness, which may inflate differences in evolutionary rates. Here, we study two phylogenetic distantly related lizard groups, which differ in the number of species, but have similar divergence times. We evaluate as follows: (1) evolutionary rate, models of evolution and phylogenetic signal among the different genital and non-genital traits; (2) which kind of traits (genital and non-genital) are divergent across sister species and (3) whether the species-rich group shows a faster rate of trait change. We studied 24 *Liolaemus* lizard species, belonging to two monophyletic groups that differ in species number: *L. elongatus'* clade, which has more species than *L. lineomaculatus'* clade. We studied 20 different traits (9 genital and 11 non-genital) and calculated their phylogenetic signal, evolutionary rate of change and models that best explain the evolutionary change. Our results show that: (1) in general, genital traits evolve faster than non-genital ones in both groups, and both phylogenetic signal and best evolutionary model vary depending on the trait. (2) Genital traits diverged more among sister species within the *L. lineomaculatus* group, but within the *L. elongatus* group, both sets of traits show similar degrees of divergence. Finally, (3) the species-rich group (*L. elongatus*), has the highest genital evolutionary rate but also the highest non-genital evolutionary rate.

Introduction

In many groups of animals, such as beetles, molluscs, fishes, snakes, lizards, and some mammals, male genitalia present morphological characteristics which have been used for systematic and taxonomic studies (Sharp & Muir, 1912; Hamilton, 1946; Gordon & Roses, 1951; Jeannel, 1955; Tuxen, 1956; Arnold, 1986a; Fitzpatrick *et al.*, 2012; Klaczko, Ingram & Losos, 2015; D'Angiolella *et al.*, 2016). In some cases, given the uniformity of some genital features, these have been used to define genera or higher taxonomic categories (Böhme, 1988; Keogh, 1999; Maduwage *et al.*, 2008; Köhler, 2009; Quipildor *et al.*, 2018a). Despite the strong phylogenetic correspondence in genital morphology, it is interesting to observe that many sister or closely related species often exhibit high divergences in some aspects of their morphology, including greater than those observed among more phylogenetically distant species

(Arnold, 1986a; Böhme, 1988; Lobo, 2000; Köhler, Dehling & Köhler, 2010; Köhler, Hahn & Köhler, 2012; Quipildor *et al.*, 2018a). Some authors have suggested that these divergences could be consequence of a high rate of change in genital features respect to non-genital features (Eberhard, 2009; Rowe & Arnqvist, 2011; Klaczko, Ingram & Losos, 2015). For example, in the genus *Gerris* (Hemiptera: Gerridae), genital features present an evolutionary pattern of rapid morphological change, compared to non-genital features (e.g., Rowe & Arnqvist, 2011).

One way to explain the faster evolution of male genitalia can be through intrasexual selection (Eberhard, 1985, 2010; Arnqvist, 1997, 1998; Hosken & Stockley, 2004; Simmons *et al.*, 2009; Klaczko, Gilman & Irschick, 2017; De-Lima *et al.*, 2019), where there is sexual competition between males- or through sexually antagonic coevolution- where the reproductive organs of males and females do not coincide adequately,

and therefore an 'arms race' between the sexes ensues (Hosken & Stockley, 2004).

Unlike most animals, males in the reptile order Squamata are characterized by having paired copulatory organs, which present a great diversity of form and ornamental structures, such as calices, flounces, and thorns (Dowling & Duellman, 1978). Despite the great diversity in this order of reptiles (~ 11.136 spp, Uetz, Freed & Jirí, 2020), there have been few comparative studies on the evolution of genitalia when compared to non-reproductive traits. In this sense, Klaczko *et al.* (2015) studied the evolutionary rates of some genital morphological characteristics in comparison with non-genital ones (six in total) in 25 species of *Anolis*. Their results suggest that the morphological characteristics related to the male genital characters evolved six times faster than the non-genital ones, possibly due to sexual selection (Klaczko & Stuart, 2015). The action of sexual selection on genital characters in both sexes might explain in part the great diversity presented by the *Anolis* genus (~442 spp., Uetz *et al.*, 2020). Following these hypotheses, it could be possible to establish a general relationship between species diversity (measured as number of species in each group), and the evolutionary rate of genital characters.

The lizard genus *Liolaemus* is one of the most diverse in the world (~278 spp., Uetz *et al.*, 2020), being the second most diverse in South America after *Anolis*. This genus is divided into two subgenera, *Liolaemus* sensu stricto or Chilean group and *Eulaemus*, or Argentinean group (Laurent, 1983), differing in their evolutionary history, which is in turn reflected in the diversity of species of the internal groups (e.g., Esquerré *et al.*, 2019). Previous work on the genus' hemipenial morphological diversity has shown a great diversity in shapes among species in each subgenus, reflecting their potential use in taxonomic and systematic studies (Lobo, 2000; Quipildor *et al.*, 2018a,b). Even though the phylogenetic relationships are well studied, until this moment no comparative studies have been made evaluating the evolution of different genital features with respect to other non-genital features and their potential relationship to the diversity of species within *Liolaemus*. Here, we study two monophyletic groups belonging of *Liolaemus* distantly related but with similar divergence time, which is an important factor when making predictions about rates of trait evolution (Harmon *et al.*, 2010): *L. lineomaculatus* group, with an approximately age of 10 Ma, and *L. elongatus*, with an approximately age of 12 Ma (see Esquerré *et al.*, 2019). We have three aims: (1) to explore the rate and models of evolution and the strength phylogenetic signal among different genital characters and compare them to different non-genital morphological characters; (2) to compare which kind of traits (genital and non-genital) have exhibited greater divergence among sibling species, and (3) to explore whether there is a relationship between the diversity of species and the rate of evolutionary change across all characters. We expect that genital characters present a faster rate of evolution change and better fit under stabilizing selection (sexual) than non-genital characters, which may present lower rate of evolution and have evolutionary patterns by Brownian motion. Additionally, as both groups have similar divergence times, we predict that the species-rich group (*L. elongatus*) has higher rate of evolutionary change in analyzed traits than group with fewer species (*L. lineomaculatus*). Furthermore, we predict that the morphological divergence in genital characteristics will be greater, with greater differentiation than non-genital characters, especially between sister species within each group. The *L. lineomaculatus* group (*Eulaemus*) consists of ~22 spp. (Breitman, Morando & Avila, 2013; Abdala & Quinteros, 2014) with a distribution restricted to the Patagonian region of Chile and Argentina (Fig. 1). In contrast, the *L. elongatus* group (*Liolaemus* sensu stricto) is more diverse (~31 spp, Ruiz *et al.*, 2019) and has a more extended distribution (from northern Argentina 26°40'29.48"S; 65°48'20.06"W. to Patagonia, Argentina 45°27'0.06"S; 69°42'0.74"W.), following the Andes mountain range, and the central region of Chile (Fig. 1). Unlike the work of Klaczko *et al.* (2015) in *Anolis*, in this work we include a larger number of characters (20 vs. 6) in two groups of lizards with similar divergence time but with a very different evolutionary history regarding its habitats and distribution.

Materials and methods

We studied genital and non-genital morphology of 24 species (10 of *L. lineomaculatus* group and 14 of *L. elongatus* group; see list of specimens in Appendix S1) from Herpetological Collection of Instituto de Bio y Geociencias del NOA (IBIGEO), Natural science museum of the National University of Salta, Salta, Argentina (MCN), Herpetological Collection of the Foundation Miguel Lillo, Tucuman, Argentina (FML), and Argentine Museum of Natural Science, Buenos Aires, Argentina (MACN). Sample sizes varied from one hemipenis to five hemipenes per species. We used small sample sizes because intraspecific variation is generally small, and these only reflect artifacts of preservation (Arnold, 1986; Böhme, 1988; Keogh, 1999). Secondly, this kind of study involves the destruction of material from museum, so a small sample size is adequate for hemipenial descriptions.

Hemipenes preparations

We following Zaher and Prudente (2003) protocol for preparing hemipenes. One hemipenis was removed from each preserved lizard via a small incision at the base of the tail. The removed organ was immersed in a 2% KOH solution for 3–5 min or until it became translucent and flexible. The hemipenis was turned outward using forceps to be sure that the whole hemipenis was fully everted. Then, the organ was completely filled with colored vaseline to allow better visualization of ornamentation structures. We obtained digital images of asulcate, and sulcate view of hemipenes using a camera (Olympus DP25) attached to a stereomicroscope (Fig. 2). Terminology of hemipenial character follow that of Dowling & Savage (1960), Savage (1997), Zaher (1999), Ziegler & Böhme (1999), Zaher & Prudente (2003), and Quipildor *et al.* (2018b). We measured all traits five times and estimated the repeatability of the measurements using the intraclass coefficient (ICC) as described by Lessells & Boag (1987) and
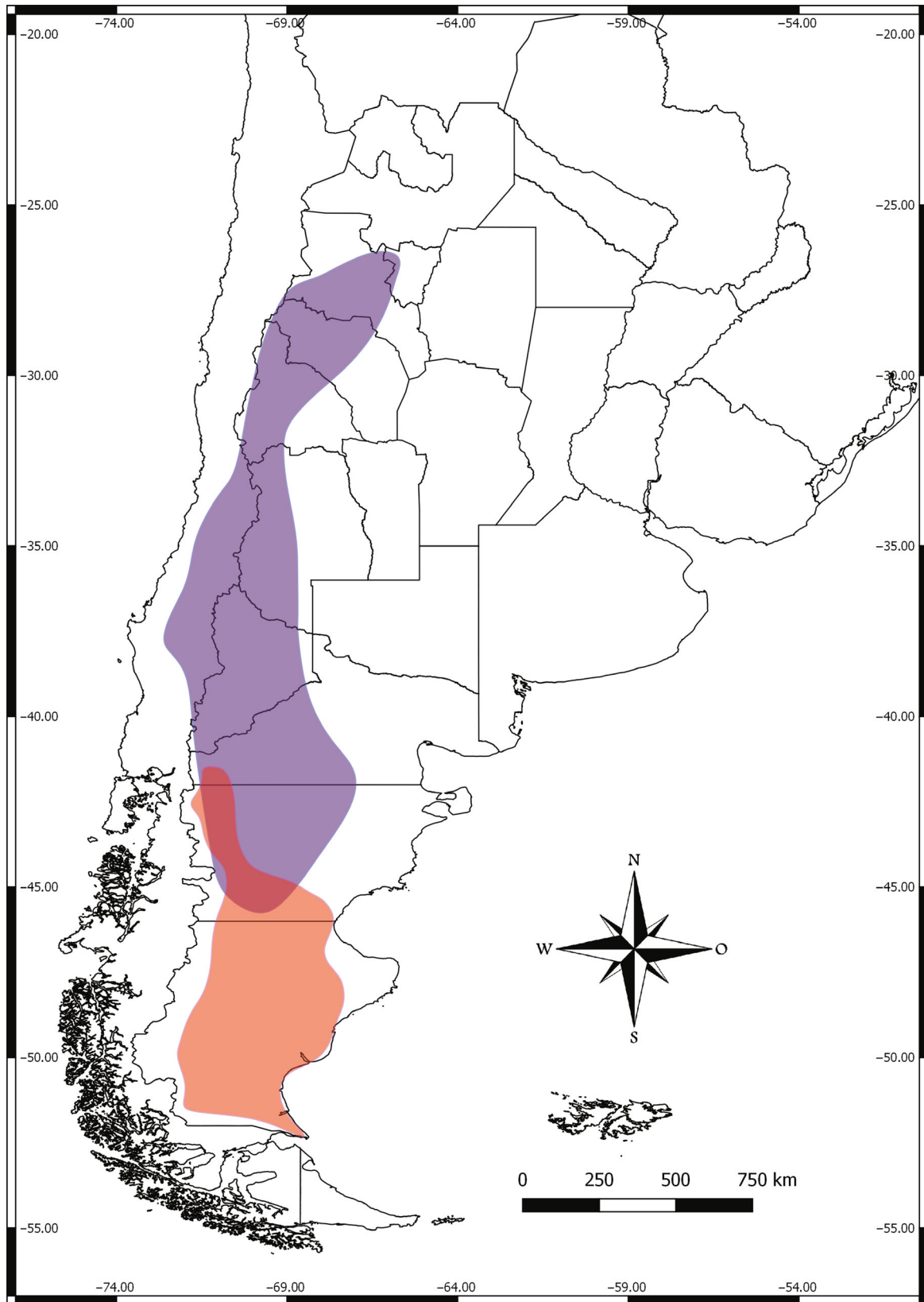
Figure 1 Distribution map of both *Liolaemus* group studied. In violet, *L. elongatus* and in pink *L. lineomaculatus* groups, respectively.
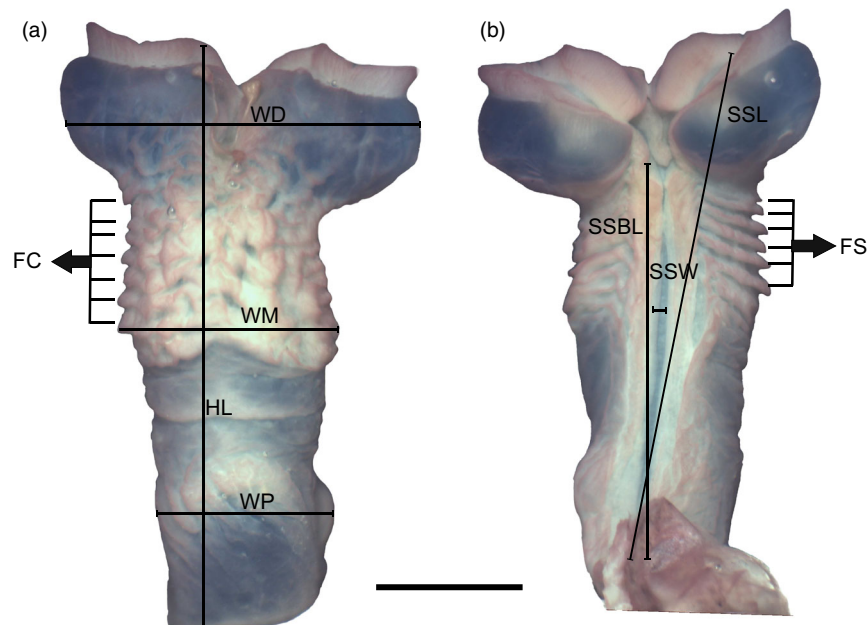
Figure 2 General figure of a typical hemipenis of *Liolaemus lineomaculatus* group: *L. kingii* (MCN 1546). Shows the genital characters used in the present study in different views: (a) asulcate face; (b) sulcate face. Hemipenial length (HL), sulcus spermaticus length (SSL), sulcus spermaticus bifurcation length (SSBL), sulcus spermaticus width (SSW), width of proximal extreme (WP), width of medial extreme (WM), width of distal extreme (WD), number of flounces in the sulcate face (FS) and fold of calyces (FC = number of transversal fold of the calyces). Scale, 2 mm.

implemented in the ICC package (Wolak *et al*., 2012) in R v 3.6.0 (R Development Core Team, 2020).

Genital and non-genital traits

We considered nine genitals and eleven non-genital traits. Following Quipildor *et al*. (2018a), we measured: hemipenial length (HL) taken from the base of the hemipenes to distal end to lobes, sulcus spermaticus length (SSL) taken from the base of the hemipenes to distal end to lobes, sulcus spermaticus' bifurcation length (SSBL) taken from the base of the hemipenes to bifurcation, sulcus spermaticus' width (SSW) taken from middle region of hemipenis, width of proximal extreme (WP) taken from base of hemipenis, width of medial extreme (WM) taken from middle region, width of distal extreme (WD) taken from distal region, number of flounces in the sulcate side (FS) and fold of calyces (FC = number of transversal fold of the calyces), see Fig. 2 for more details. The non-genital traits studied were: forelimb length (FoL), trunk length (TL; distance from axilla to anterior edge of hindlimb insertion), foot length (FL), tibia-fibula length (TFL), radius-ulna length (RUL), hand length (HAL; from proximal extreme until longest digit), head height (HH), head width (HW), head length (HL), auditory meatus height (AH). For the *L. elongatus* group, the genital and non-genital traits were taken from 78 male individuals representing 14 species (Appendix S1). For the *L. lineomaculatus* group, we analyzed the genital traits of 31 male individuals, representing 10 species (Appendix S1). The non-genital traits of the *L. lineomaculatus* group were taken from Breitman *et al*. (2013) study. The

measurements of genital and non-genital traits were taken using a digital caliper (0.02 mm precision), and an ocular micrometer. In addition, we measured its snout-vent length of each specimen to use as a covariate in subsequent statistical analyses.

Statistical analyses

We performed comparative analyses using the Liolaemidae phylogenetic tree of Esquerré *et al*. (2019). We extracted *L. lineomaculatus* and *L. elongatus* groups, using 'drop.tip' and 'extract.clade' functions, in the 'ape' package (Paradis, Claude & Strimmer, 2004). Prior to the analysis, we obtained the average per species for each measurement (genital and non-genital traits) and performed a phylogenetic size correction to eliminate the body size dependence. For this, we use the phytools package and the function phylo.resid (Revell, 2009) obtaining, the residuals from a least squares regression. These residuals were used in subsequent analyses as size-corrected morphological measurements.

First, we estimated the degree of phylogenetic signal for each genital and non-genital trait. We calculated Blomberg's K (using phylosig in package Phytools; Revell, 2012) values. Phylogenetic signal is a measure of the tendency of related species to resemble one another and Blomberg's K is a quantitative measure of this pattern (Blomberg *et al*., 2003). Those K values lower than 1 and closer to 0 indicate little or no phylogenetic signal associated to a random or convergent evolution of trait. On the other hand, those K values that are closer, equal or greater than 1, suggest stronger similarities among

closely related species and thus indicates a substantial degree of trait conservatism (Blomberg *et al.*, 2003). Secondly, employing Adams' likelihood models (Adams, 2013), we obtained and compared the values of evolutionary change rate (σ^2) between both groups. This was done running 'compare.evol.rates' a function of 'geomorph' package (Adams *et al.*, 2013). Thirdly we explored three possible models of evolutionary change that could be acting on these traits: 1- Brownian Motion evolutionary model (BM), where the evolutionary change in a trait occurs as result from random fluctuations through time (Felsenstein, 1988; Harmon *et al.*, 2010). 2- Ornstein–Uhlenbeck model (OU), where the trait varies in relation to one or more evolutionary optima, at least in part of the lineage (Hansen, 1997; Butler & King, 2004) and finally 3- Early Burst (EB), in the case that a trait changes early in the evolutionary tree and then the rate of evolution slows down gradually. We tested, which of these three evolutionary models fitted better with different genital and non-genital traits (Appendix S2). These analyses were done running the fitContinuous function using the geiger (Harmon *et al.*, 2008) and 'ape' packages (Paradis *et al.*, 2004). We used Akaike's criterion corrected for small sample size (AICc) to select the best fit models (Appendix S2), which has the lowest value for AICc and the highest Akaike's weight (AICc*W*; Burnham & Anderson, 2004).

To visualize those traits that have more variability and divergence, we performed a phylogenetic Principal Component Analysis (pPCA), including both genital and non-genital traits. For this, we implemented 'phyl.pca', function of 'phytools' package, which is based on variance–covariance matrix using a Varimatrix rotation (Revell, 2012). We interpreted the patterns of covariation among traits in relation to the phylogenetic affinities of the species with a phylomorphospace plot, using the function 'phylomorphospace', based on the scores from the first four principal components axes. In addition, to have a better comprehension about the importance of phylogenetic relationships among species on amount of variation explained by a PCA, we performed a principal component analysis to compare our results using 'prcomp', a function of 'stats' package (see Appendix S3).

Finally, to know whether there is an association between higher values of evolutionary rate and the number of species of both groups, we performed a non-parametric Wilcoxon signed-rank test, using evolutionary rates from both sets of traits (genital and non-genital), and each clade (*L. lineomaculatus* = fewer species; *L. elongatus* = more species) as a group, following by their descriptive statistics. For these, we used 'wilcox.test', a function of 'stats' package. As hemipenial studies involve the destruction of specimens from museum, we cannot represent the totality of species diversity in both groups. Because of this we have limited coverage of the diversity of species (48% and 45% for *L. lineomaculatus* and *L. elongatus* groups, respectively), which may induce a bias due to taxon sampling (Garamszegi & Møller, 2010). For this, we performed a species sensitivity test to evaluate this effect, for detail see Appendix S4. All analyses were performed using R v 3.6.0 (R Development Core Team, 2020).

Results

Measurements of genital traits were repeatable: hemipenial length (ICC = 0.75), sulcus spermaticus length (ICC = 0.73), sulcus spermaticus bifurcation length (ICC = 0.73), sulcus spermaticus width (ICC = 0.68), width of proximal extreme (ICC = 0.53), width of medial extreme (ICC = 0.58), width of distal extreme (ICC = 0.53), all with a *P* value <0.05.

Table 1 shows *K* values of genital and non-genital traits of both, *L. lineomaculatus* and *L. elongatus*. Two traits had values of *K* exceeding 1.0, length of sulcus spermaticus and sulcus spermaticus bifurcation lengths (Fig. 2) suggest a strong phylogenetic inertia in these traits, as opposed to sulcus spermaticus width (in both, *L. lineomaculatus* and *L. elongatus* groups) and proximal width of hemipenes (only in *L. elongatus* group) which were lower than one. The other genital traits: medial extreme width, distal extreme width and the number of flounces in the sulcate and asulcate faces have low *K* values in both groups, suggesting a weak phylogenetic signal in these characters. With respect to non-genital traits, both groups have low *K* values for all characters, with the exception tibia-fibula length, hand length, and head height in *L. lineomaculatus* and radio-ulna length in both groups.

The comparative analysis of evolutionary rates using square sigma (σ^2) values, showed that *L. elongatus* group had significant differences in the σ^2 values with respect to *L. lineomaculatus* group (Table 1). The σ^2 values of: sulcus spermaticus width and both: width of proximal extreme and the number of flounces in the sulcate face in the *L. elongatus* group were higher than *L. lineomaculatus* group (Table 1). With respect to non-genital traits, in almost all of them the values of σ^2 showed significant differences (Table 1). Whereas in *L. elongatus* group were higher than in *L. lineomaculatus* group (Table 1).

Evolutionary models test (Appendix S2) showed that in general, genital traits in *L. lineomaculatus* group had better fit under a BM evolutionary model (see Table 1). In contrast, the width of medial extreme and the number of transversal folds of the calyces, showed a better fit under OU evolutionary model (Table 1). Whereas in *L. elongatus* group had better fit under an OU evolutionary model, which suggests that these traits may evolve under the action of one of more selective forces (see Table 1). In contrast, hemipenial length and sulcus spermaticus length, showed a better fit under BM evolutionary model. Other as the width of distal extreme, showed a double fit BM/OU in both groups (Table 1). Non-genital traits showed variable best fit in *L. lineomaculatus* group: mainly BM, and secondarily OU or double fit, whereas in the *L. elongatus* group mainly OU (Table 1).

Considering principal component analyses, phylogenetic and ordinary (Appendix S3 for this latter) approaches, both provided similar results. However, the ordinary PCA, with a phylogenetic bias, as we did not consider the relationship among sister species, showed less percentage of variability among traits (i.e., less variability among sister species; Appendix S3) had) than phylogenetic analyses. The first four axes from the pPCA analysis accounted for 90.8% and 91 % of the total

Table 1 Comparisons of morphological traits between both *Liolaemus* groups (*L. lineomaculatus* = *L. lin.*; and *L. elongatus* = *L. elon.*), considering genital and non-genital traits and showing: K (values of phylogenetic signal) and those *P*- values associated and the rate of evolutionary change or σ^2 and its $P(\sigma^2)$ values associated to test the difference between both clades

Traits	$K_{L.\ lin.}$	$P_{L.\ lin.}$	$K_{L.\ elon.}$	$P_{L.\ elon}$	$\sigma^2_{L.\ lin.}$	$\sigma^2_{L.\ elon.}$	$P(\sigma^2)$	BEM $_{L.\ lin.}$	BEM $_{L.\ elon.}$
Genital									
HL	1.26	0.01	0.94	0.004	0.21	0.15	0.58	BM	BM
SSL	1.36	0.01	0.93	0.02	0.12	0.15	0.72	BM	BM
SSBL	1.17	0.01	0.93	0.004	0.09	0.13	0.55	BM	BM
SSW	0.4	0.53	0.21	0.62	0.0005	0.003	**0.003**	BM/OU	OU
WP	1.72	0.01	0.47	0.1	0.01	0.03	**0.04**	BM	BM/OU
WM	0.23	0.94	0.47	0.1	0.62	1.93	0.06	OU	BM/OU
WD	0.46	0.37	0.47	0.6	0.086	0.09	0.90	BM/OU	BM/OU
FS	0.66	0.22	0.36	0.25	0.62	1.93	0.06	BM	OU
FC	0.3	0.84	0.14	0.83	0.30	6.22	**0.01**	OU	OU
Non-genital									
FoL	0.3	0.35	0.28	0.5	0.05	1.31	**0.001**	BM/OU	OU
TL	0.48	037	0.27	0.41	0.23	2.75	**0.001**	BM/OU	OU
FL	0.41	0.54	0.24	0.5	0.16	0.96	**0.01**	OU	OU
TFL	1.5	0.004	0.23	0.58	0.08	0.60	**0.01**	BM	OU
RUL	0.75	0.05	0.86	0.01	0.03	0.10	**0.05**	BM/OU	BM/OU
HAL	0.7	0.18	0.48	0.1	0.04	0.42	**0.01**	BM	OU
HH	1.4	0.005	0.3	0.38	0.03	0.10	0.06	BM	OU
HW	0.48	0.38	0.38	0.2	0.02	0.14	**0.01**	BM/OU	OU
HL	0.58	0.18	0.24	0.53	0.06	0.45	**0.02**	BM/OU	OU
AH	0.36	0.68	0.17	0.72	0.008	0.10	**0.001**	OU	OU

Genital traits: hemipenial length (HL), sulcus spermaticus length (SSL), sulcus spermaticus bifurcation length (SSBL), sulcus spermaticus wide (SSW), width of proximal extreme (WP), width of medial extreme (WM), width of distal extreme (WD), number of flounces in the sulcate face (FS), number of transversal fold of the calyces (FC). Non-genital traits: forelimb length (FoL), trunk length (TL), foot length (FL), tibia-fibula length (TFL), radius-ulna length (RUL), hand length (HAL), head height (HH), head width (HW), head length (HdL) and auditory meatus height (AH).
Values of $P(\sigma^2)$ that are statistically significant ($P < 0.05$) are in boldface.
Best evolutionary model (BEM): Brownian Motion (BM), Ornstein–Uhlenbeck (OU).

variance in *L. lineomaculatus* and *L. elongatus* groups, respectively (Tables 2 and 3). Figure 3 shows the percentage of variation accounted for the four by more explained axes in both groups studied here. In *L. lineomaculatus* group, six genital traits (hemipenial length, sulcus spermaticus length, sulcus spermaticus bifurcation length, sulcus spermaticus width, number of flounces in the sulcate side, number of transversal folds of calyces) and three non-genital traits (forelimb length, foot length, head width) were the greatest contributors to PC1 (48.91%; Fig. 3; Table 2). The second axis (PC2 20.79 % Fig. 3a; Table 2), was mostly explained by the width of medial and distal extremes of hemipenes and the trunk length. The third axis (PC3 14.66%; Fig. 3b; Table 2) was explained by three non-genital traits: radius-ulna length, hand length, head height. The fourth axis (PC4 6.44 %; Fig. 3c; Table 2) was explained by tibia-fibula length.

On the other hand, for *L. elongatus* group, the first axis (PC1 42.88 %; Fig. 3; Table 3) had two genital traits (sulcus spermaticus width, number of flounces in the sulcate side) and five non-genital traits (trunk, foot, tibia-fibula lengths, width and length of head) as more contributed traits. The second axis (PC2 22.41%; Fig. 3d; Table 3) was explained by the number of transversal folds of the calyces. The third axis (PC3 18.06 %; Fig. 3e; Table 3) was explained by two genital (width of

proximal and medial extremes) and two non-genital (forelimb length, head height) traits. Finally, the fourth axis (PC4 7.65 %; Fig. 3f; Table 3) was explained by third genital traits: hemipenial length, sulcus spermaticus length, and sulcus spermaticus' bifurcation length.

Wilcoxon signed-rank test (Table 4) showed that the rates of evolution in genital traits exceeded the rates for non-genital. In addition, rates of evolution in both sets of traits were greater in *L. elongatus* than *L. lineomaculatus* ($T = 6$, $Z = 1.95$; $P = 0.05$) and non-genital ($T = 0.004$, $Z = 2.80$; $P < 0.01$) traits. Results of sensitivity species analyses (Appendix S4) showed that a representativity of 25 and 75 % may have differences respect 100%, whereas between 50 % and our representativity (48% *L. lineomaculatus* and 42% *L. elongatus*) did not in both analyzed group to analyze intragroup (Appendix S4). Whereas intergroup analysis showed that patterns are conserved in all percentages, including our representability. Thus, our analysis would be similar those made considering the total of species represented (Appendix S4).

Discussion

From the comparative analysis of genital characters between groups, we observed a great heterogeneity regarding the

Table 2 Traits' contributions for each axis in the *Liolaemus lineomaculatus* group

Traits	PC1 (48.91%)	PC2 (20.79%)	PC3 (14.66 %)	PC4 (6.44 %)
Genital				
HL	**−0.745**	−0.479	0.418	−0.173
SSL	**−0.749**	−0.469	0.439	−0.078
SSBL	**−0.805**	−0.402	0.422	−0.027
SSW	**−0.728**	0.028	−0.006	0.487
WP	−0.446	−0.070	0.2467	0.058
WM	−0.018	**−0.638**	−0.379	−0.013
WD	0.13	**0.719**	0.323	−0.395
FS	**−0.896**	0.351	−0.243	−0.112
FC	**−0.829**	−0.033	0.223	0.179
Non-genital				
FoL	**0.723**	−0.095	0.124	−0.434
TL	−0.078	**−0.779**	−0.480	−0.124
FL	**0.861**	0.041	0.0124	−0.450
TFL	0.197	−0.035	−0.513	**0.653**
RUL	0.285	−0.503	**0.648**	−0.181
HAL	0.501	0.349	**−0.675**	−0.294
HH	−0.048	−0.395	**0.738**	0.286
HW	**0.780**	0.338	0.301	0.013
HdL	0.339	0.398	−0.606	0.325
AH	−0.531	−0.278	−0.196	0.409

Genital traits: hemipenial length (HL), sulcus spermaticus length (SSL), sulcus spermaticus bifurcation length (SSBL), sulcus spermaticus width (SSW), width of proximal extreme (WP), width of medial extreme (WM), width of distal extreme (WD), number of flounces in the sulcated face (FS), number of transversal fold of the calyces (FC). Non-genital traits: forelimb length (FoL), trunk length (TL), foot length (FL), tibia-fibula length (TFL), radius-ulna length (RUL), hand length (HAL), head height (HH), head width (HW), head length (HdL) and auditory meatus height (AH).
In bold those with a higher contribution (≥ [0.60]) to the axes more relevant in function of their percentage of variation (90.8 % in total)

Table 3 Traits' contributions for each axis in the *Liolaemus elongatus* group

Traits	PC1 (42.88%)	PC2 (22.41%)	PC3 (18.06%)	PC4 (7.65%)
Genitals				
HL	0.323	0.251	−0.167	**−0.642**
SSL	0.158	0.082	−0.013	**−0.696**
SSBL	0.072	0.130	0.128	**−0.707**
SSW	**−0.721**	0.286	0.278	−0.194
WP	0.091	0.262	**−0.647**	0.163
WM	0.225	0.295	**−0.718**	−0.001
WD	0.336	0.237	−0.520	−0.570
FS	**0.857**	0.421	−0.124	0.140
FC	0.553	**0.780**	0.136	−0.064
Non-genital				
FoL	0.296	−0.181	**−0.896**	0.240
TL	**0.796**	−0.415	0.383	0.172
FL	**0.678**	−0.385	−0.115	−0.550
TFL	**0.600**	−0.522	−0.415	−0.055
RUL	−0.008	−0.193	−0.220	0.572
HAL	0.356	−0.513	0.497	−0.203
HH	0.360	−0.120	**−0.778**	0.047
HW	**0.752**	−0.146	−0.320	−0.382
HdL	**0.621**	−0.217	−0.523	−0.405
AH	0.561	−0.140	−0.685	0.113

Genital traits: hemipenial length (HL), sulcus spermaticus length (SSL), sulcus spermaticus bifurcation length (SSBL), sulcus spermaticus width (SSW), width of proximal extreme (WP), width of medial extreme (WM), width of distal extreme (WD), number of flounces in the sulcated face (FS), number of transversal fold of the calyces (FC). Non-genital traits: forelimb length (FoL), trunk length (TL), foot length (FL), tibia-fibula length (TFL), radius-ulna length (RUL), hand length (HAL), head height (HH), head width (HW), head length (HdL) and auditory meatus height (AH).
In bold those with higher contribution (≥ [0.60]) axes more relevant in function of their percentage of variation (91 % in total)
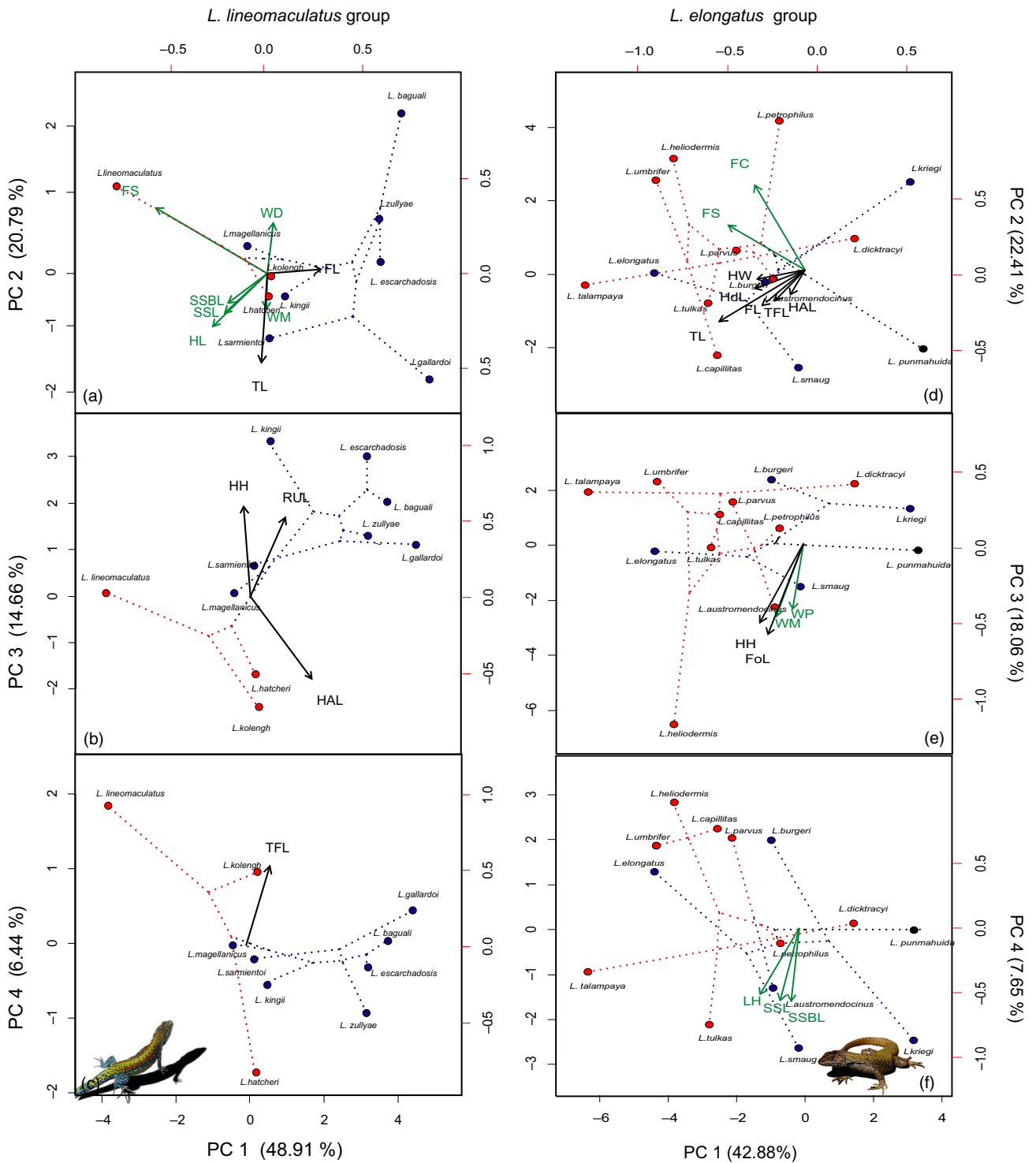
Figure 3 Biplot of the phylogenetic principal component analysis (pPCA) for the four principal components (PCs) of *Liolaemus lineomaculatus* and *L. elongatus* group, respectively. (a-d) First and second, (b-e) First and third, (c-f) First and fourth. Green arrows indicate genital traits, whereas black arrows indicate non-genital traits. The percentages of variance explained by PCs are shown in the axis labels.

evolution of these characters. In both groups, the genital traits seem to evolve independently and faster than corporal (non-genital) traits, in a similar manner to what were suggested by

other authors for many groups of animals, including lizards (Arnqvist, 1997; Hosken & Stockley, 2004; Eberhard, 2009, 2010; Rowe & Arnqvist, 2011; Klaczko, Ingram & Losos,

Table 4 Wilcoxon signed-rank test results showing the comparison between the evolutionary rate values in genital and non-genital traits in both studied group: *Liolaemus lineomaculatus* (fewer species) and *L. elongatus* (more species)

Traits	T	Z	P	Mean $\pm$ SD	
				L. lineomaculatus (<spp.)	*L. elongatus* (>spp.)
Genital	6	1.95	0.05	0.23 $\pm$ 0.24	1.18 $\pm$ 2.05
Non-genital	0.004	2.80	<0.01	0.07 $\pm$ 0.24	0.70 $\pm$ 2.05

Additionally, we showed the mean values $\pm$ standard deviation (SD) of evolutionary rate values.

2015; Klaczko & Stuart, 2015). With respect to phylogenetic signal, the traits: width of sulcus spermaticus, width of medial, and distal extreme and number of flounces on the sulcate and asulcate side showed little phylogenetic inertia in their evolution. These patterns may be due to intersexual selection or sexually antagonistic coevolution of genital morphology (Eberhard, 1985, 2010; Arnqvist, 1997, 1998; Hosken & Stockley, 2004; Simmons *et al.*, 2009; Klaczko, Gilman & Irschick, 2017; De-Lima *et al.*, 2019), where females might present certain traits that benefit the transfer of sperm (lock and key hypothesis; Dufour, 1844; Masly, 2012). On the other hand, genital characters such as hemipenis length, sulcus spermaticus length and its bifurcation, or proximal width (only in *L. lineomaculatus*), had a high phylogenetic signal, suggesting a greater historical inertia in its evolution, similar to what is suggested in other groups of animals such as formicids (Song & Bucheli, 2010). These type of characters with high phylogenetic signal would be relevant in systematic studies, as is suggested by different studies in Squamata and other groups of animals with intromitent organs (Sharp & Muir, 1912; Hamilton, 1946; Gordon & Roses, 1951; Jeannel, 1955; Tuxen, 1956; Higgins, 1975; Arnold, 1986a; Fitzpatrick *et al.*, 2012; Köhler, Hahn & Köhler, 2012; Klaczko, Ingram & Losos, 2015; Quipildor *et al.*, 2018b).

From the phylogenetic PCA analysis, and ordinary PCA (Appendix S2), we observed that in both groups the genital and the non-genital traits showed great divergences between sister species or very closely related species considering the more explaining axes. Nevertheless, the *L. lineomaculatus* group shows greater divergence in genital traits compared to the non-genital ones (8 and 4, respectively, see Table 2). On the other hand, the species of the *L. elongatus* group presented greater divergence in the non-genital traits (5 traits, Table 2) than in the genital ones (3 traits, see Table 3). A recent study on the genital morphology in a phylogenetic context in *Liolaemus*, showed similar divergences between pairs of closely related species, which suggests a fundamental role in the hemipenial characters in the reproductive isolation (Quipildor *et al.*, 2018a). Our results add evidence to the hypothesis that genital and non-genital traits evolve differently and independently in both groups (Fig. 3; Tables 2 and 3). Based on this, several questions arise, such as: why don't species of the *L. elongatus* group present as much differentiation in genital traits as the *L. lineomaculatus* group? A possible answer may be that sexual barriers (genital characters) would not be necessary, given that they present a higher divergence in non-genital traits, accompanied by a higher evolutionary rate. In fact, all non-genital traits in the *L. elongatus* group (with the exception of

radius-ulna length) presented low phylogenetic signal values, this indicated that could be under selective forces. The low divergence in genital morphology in the *L. elongatus* group could explain the hybridization processes between sympatric species, as was hypothesized by Troncoso-Palacios et al. (2019). Although is important to mention that in general *L. elongatus* group exhibits highly evolutionary patterns (Olave *et al.*, 2020).

Finally, the differences in the evolution of genital and non-genital traits in each group might translate into the diversity of the group *per se*. The least diverse group, *L. lineomaculatus* (~22 spp; Breitman *et al.*, 2013), exhibited lower evolutionary rate in genital and non-genital morphology than the *L. elongatus* group, which has more species (~31 spp Ruiz *et al.*, 2019; Table 4). Our results show that in general, genital characters evolve at a faster rate than non-genital characters. These results are similar to Klaczko's study of *Anolis* lizards (Klaczko, Ingram & Losos, 2015; Klaczko, Gilman & Irschick, 2017; Klaczko & Stuart, 2015).

Without a doubt, intromitent male genitalia can be conducive to evolutionary studies of complex morphological traits. Given the little background on the subject and the complexity of these systems, the present work gives us more questions than answers. Therefore, in future studies we propose to tackle other aspects that were not taken here into account. For example, we intend to further investigate whether the variations found in the genitals act as a reproductive barrier, as the key and lock hypothesis suggests, and whether this hypothesis explains the diversity in species of the *L. elongatus* group. Finally, we intend to deepen our understanding of the evolution of these sets of traits and broaden our scope to a phylogenetically more inclusive group, such as Liolaemidae or even Squamata.

Conclusions

Our results show that genital traits present different values of phylogenetic signal, evolutionary models, rates, and ways within and between clades. Considering the principal component analysis (phylogenetic and common), the genital characters in the *Liolaemus lineomaculatus* group contributed the most to the variability and divergence between sister species or closely related species. On the other hand, in the *L. elongatus*, group, non-genital traits were more divergent. Finally, genital traits on the whole show a higher evolutionary rate with respect to the non-genital traits. The *L. elongatus* group (which has more species) presented higher evolutionary rates in both genital and non-genital traits. It is interesting see that there is

a similar pattern between the species studied in this work (from the *Liolaemus* genus) and the species of the *Anolis* genus studied by Klaczko *et al.* (2015), despite their differences in life history, phylogeny and diversification patterns. That is the evolutionary rates in genital traits tend to be higher than the non-genital ones.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. List of specimens studied for morphological analysis.

Appendix S2. Evolutionary models test

Appendix S3. Ordinary principal component analyses.

Appendix S4. Sensitivity analysis.